AN EXPERIMENTAL STUDY OF ACCLIMATION TO TEMPERATURE IN PLANARIA DOROTOCEPHALA.

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I. INTRODUCTION.

It is a familiar fact that within certain limits within which the organism can continue to function normally, metabolic rate varies directly with temperature. As far back as 1865, Sachs (1865) studied the effect of rise in temperature upon a great number of biological phenomena and with a great variety of material, and came to that general conclusion. Very accurate data upon the rate of growth were obtained by Féré (1894) working with hen's eggs; upon rate of activity of protoplasm by Nägeli (1860), Schultze (1863), Hofmeister (1867), and others, and especially by Velten (1876) who made an accurate quantitative determination of the effect of temperature upon the activity of chlorophyll grains in *Elodea*, *Vallisneria* and *Chara;* upon oxygen absorption, by Treviranus (1831) working on the honey-bee; upon carbon dioxide excretion, by Rossbach (1872) in studies on the contraction rate of vacuoles in various protozoa; and

upon these and other phases of the subject by many other later investigators. The general conclusions on the direct effect of temperature may be summed up in the words of Verworn (1899): "Within certain limits increasing temperature acts to augment vital processes. Up to a certain point excitation increases with increase of temperature. This holds good for very different phenomena and for very different forms of living substance."

But the phenomenon of acclimation to temperature changes, while well known, has not been submitted to extensive analysis. It has been observed of course that in warm springs organisms are found, living at temperatures as high as 85° C. (Flourens, 1846), or even 98° C. (Ehrenberg, 1859), closely related to species which live in water seldom as high as 40° C. Acclimation to temperature has also been observed in *Euglena*, where Schwartz (1884) and Aderhold (1888), working separately, found that *Euglena* collected in summer are not active below 5° or 6° C., in winter at as low as 0° C. No explanation for this phenomenon is offered and no further experiments have been performed upon this material. But Dallinger (1880) found that flagellates will endure a rise of temperature from 15.6° C. to 70° C. if the change is very gradual. And both Schottelius (1867) and Dieudonné (1894) succeeded in causing bacilli which normally produced a fluorescing pigment and trimethylamine at 22° and not at 35°, to produce these substances at 35° if kept at that temperature through a sufficient number of generations.

The most complete and elaborate experiments on vertebrates are those of Davenport and Castle (1895) upon *Bufo* tadpoles, and those of Loeb and Wasteneys (1912) upon *Fundulus*. Davenport and Castle found that *Bufo* tadpoles kept at 15° went into heat rigor at 40.3° C., from which state recovery was possible; but those kept at 25° C. for 28 days resisted this higher temperature perfectly, going into heat rigor only at 43.5°. If brought back to 15° for 17 days they lost their resistance to high temperature, but only partially, going into heat rigor now at 41.6° C. The *Fundulus* experiments of Loeb and Wasteneys showed similar results. Fish from a temperature of 10° C. die in less than two hours at 29° C., and in a few minutes at 35° C.; but if first exposed to 27° C. for 40 hours they can live indefinitely at 35° C., and

may even endure 39° if the rise is gradual. This acquired resistance to high temperature persists through four weeks' subsequent exposure to very low temperature. In these two cases an alteration of temperature has evidently produced a persistent effect on the protoplasm.

All the observations and experiments quoted show that organisms have the capacity to acclimate to considerable changes of temperature if these are brought about gradually; but very slight attempts have been made to give an explanation of the physiological significance of the acclimation process. The only noteworthy attempt to analyze the processes involved in acclimation is that of Davenport (1897). In regard to the Confervæ that live in hot springs, he suggests the following: Since it is known that the coagulation point of at least one protein, egg albumin, rises in proportion as it is dried, and since a number of investigators have held that death from high temperature is due to coagulation of proteins, the increased resistance to extreme heat is probably due to loss of water. However, there is some evidence at present that death from high temperature is due rather to accumulation of acids in the tissues (Mayer, '17), which throws some doubt upon Davenport's interpretation of acclimation in hot springs. Further, acclimation to extreme cold is, according to Davenport, a loss of irritability. The process of acclimation, then, consists in the modification of protoplasm through excessive heat or cold in such a way that it is not so strongly irritated by these extreme temperatures, and that the coagulation and freezing points are shifted, possibly through loss of water (1897, p. 258). This conception of the process of acclimation is inadequate in that it regards protoplasm as a static mass, which alters its condition through successive stages, rather than as a continually changing dynamic system.

The studies reported in this paper were undertaken to determine the effect of temperature changes upon the metabolism of *Planaria dorotocephala* and to discover if possible a physiological basis for the phenomenon of acclimation to temperature changes.

The work was carried on at the University of Chicago, 1915–1918, at the suggestion and under the direction of Prof. C. M.

Child. It is a great pleasure to me to have this opportunity to express my thanks to Professor Child, not only for the stimulus of his ideas and for his valuable suggestions, but for his kindness in placing at my disposal certain unpublished data from his own experiments concerning the effect of temperature on head-frequency and head-form in the regeneration of pieces of *Planaria*. My warm thanks are due to Dr. L. H. Hyman, of the Department of Zoölogy of the University of Chicago, for her keen and thoughtful criticism, constant encouragement, and constructive advice. I wish further to acknowledge my deep indebtedness to other colleagues and to the friends whose help during these three years has made effort easy.

II. Material, General Methods and Terminology.

The material used for these experiments was *Planaria dorotocephala*, one of the triclad turbellarians, the same species used extensively by Child ('11, *a*, *b*, *c*, etc.) in his earlier studies on the axial metabolic gradient. It is found in the springs that feed into swamps a short distance back from the banks of the Fox River, near Cary, Ill. The material lends itself very well to temperature experiments; for though its natural habitat is in waters of relatively low temperature, the stock lives readily in the laboratory at 17° C., and can continue to exist to all intents normally in temperatures varying from somewhat over 30° C. to at least as low as 4°. The temperatures employed all lay well within these limits, between 30° and 5°. The stock was collected and brought into the laboratory at intervals during the time covered by the experiments, and was fed on liver three times weekly (the frequency which has been found necessary to maintain growth) throughout the period.[1] With this treatment the stock maintains itself in normal condition.

For the experimental work three general temperatures were employed, approximately 10° apart; that of the refrigerator ranging between about 8° and 10° C., that of the general laboratory, 18° to 20°; and that of a warm chamber, between 27° and

[1] During a part of the time high temperature stock was fed more often, as it was found that metabolism is so rapid at raised tmperature on tri-weekly feedings that the worms not only may not grow but may even decrease in size.

30° C. For convenience these will be called "low," "medium" and "high," and the exact temperatures given only when this seems significant for the purpose of the experiment. Several other terms used should be explained here. By "living" temperature is meant, not the temperature of the natural environment, but that temperature at which they have been living for a certain experimental period; in acclimation experiments there is an "acclimation temperature." "Regulation temperature" means that temperature to which the worms are subjected during regulation; similarly, "testing temperature" means the temperature at which the metabolic condition of the worms is tested. When two temperatures are given, the first is the living, the second the testing or regulating temperature.

The general methods employed in estimating the effect of temperature upon the metabolism were:

1. Susceptibility.
2. Measurements of CO_2 production by
    1. Colorimetric method.
    2. The biometer.
3. Rate of regulation and head-frequency.

These methods will be described more fully as the experiments are reported.

III. General Observations.

Even before any actual experiments are attempted, certain general observations on the effect of temperature upon the worms can be made. Out-of-door stock lives at a temperature of not more than 8–14°. Under these conditions the worms are not very active, rather small but stocky, and of dark color. When brought into the laboratory and kept there under temperature conditions approximately like those out-of-doors they maintain the same general appearance and are very sluggish even in response to light. When kept at ordinary room temperature, however, which during most seasons of the year represents a rise of at least 7–8° C., the stock becomes more active and loses some of its heavy pigmentation, changes which we have come to associate with more rapid metabolism. Even if well fed the worm shows these changes; and they are much more marked in stock that is put at still higher temperature. At 27–30° C.

the metabolic rate is so high that it is difficult to keep stock sufficiently nourished to allow it to increase in size. The worms are restless, and soon become thinner, narrower and much lighter in color. For example, a stock collected November 17, 1917 and set in the warm chamber January 11, 1918 at average temperature of 27.5° was fed tri-weekly. The reduction in size was rapid; the worms originally 15–18 mm. in length decreased within a period of two weeks to 9–10 mm., and were very much more slender than the worms of the same stock and size in the same temperature under daily feeding. It was further noticed, though no measurements were taken, that this reduction was more rapid, even, than in a parallel starvation stock at lower temperatures—ranging from 14–16°. That these differences in appearance and behavior are directly associated with the metabolic rate needs no further proof. Even the pigment changes cannot be a matter of kind of food but must, it seems, be related to oxidation rate in some such way as the alterations in pigmentation which cause seasonal dimorphism in butterflies (Dorfmeister, 1879, Weismann, 1895, et al.).

IV. Relation of Susceptibility to Temperature Changes.

The experiments to be reported in this section deal with alterations of metabolic rate in acclimation as tested by the susceptibility method. This method as devised by Child ('13*a*) consists in subjecting the animals to concentrations of certain agents which will kill them slowly enough to permit one to observe accurately differences in their time of death. Child has sufficiently demonstrated that the time required for death is dependent upon metabolic rate, being shorter the higher the rate. Therefore in my experiments I have used the time required for death as a measure of the effect of various conditions of temperature upon the metabolic rate.

In all the experiments reported here, KNC was the agent used. The work of earlier physiologists, notably that of Geppert (1889), demonstrated that the action of KNC on vertebrates in some way prevents the tissues from utilizing the oxygen of the blood. Loeb and various other more recent workers have used KNC extensively to inhibit oxidations; and it has been shown

that cyanides decrease the activity of oxidizing enzymes. Hyman ('16) has recently shown that in all except very low concentrations cyanides decrease oxygen consumption in the sponge. I am also permitted to mention the results of recent experiments on *Planaria dorotocephala* not yet published: Dr. Hyman shows that here also cyanides decrease oxygen consumption to a marked degree, and Professor Child has been able to demonstrate a parallel decrease in CO_2 production in the same material under the influence of cyanides. This action of KNC upon oxidation makes it very effective for the purposes of these experiments, since metabolic rates are best measured in terms of oxidative processes. And Child ('13*a*) has presented evidence of the fact that susceptibility to cyanide increases with rise in temperature, so there is good precedent for the use of this agent.

The concentration of KNC found most effective was a 1/1,000 molecular solution made up with water of the appropriate temperature. In each lot 10 worms of as nearly as possible the same size were used. They were put in a 100 c.c. Erlenmeyer flask, the water drained off and the worms then rinsed in the appropriate cyanide solution (made up fresh each time), after which the flasks were filled and stoppered tightly to prevent loss of KNC by evaporation. The method of recording death rates was that employed by Child ('15) in which certain arbitrarily defined stages in the course of disintegration were distinguished as follows:

Stage I.—The worm is still intact.

Stage II.—The first signs of disintegration are apparent. These usually appear at the head end but, as we would expect, also very soon in the region of the posterior zooids.

Stage III.—The beginning of disintegration on the margins posterior to the head.

Stage IV.—Margins completely disintegrated.

Stage V.—There is no tissue left with any appearance of life.

From the data thus obtained graphs were plotted by the method that Child has previsouly used ('15, p. 81).[1]

[1] This method consists in giving numerical values to the stages of disintegration as follows: Stage II., 1; Stage III., 2; Stage IV., 3; Stage V., 4; and using as

A. *Long Time Acclimations.*

In the first series of experiments, stocks of worms were used which had been in the three temperatures for periods of time from three days to three months. The susceptibility of lots of worms from each of these stocks was then tested at a different

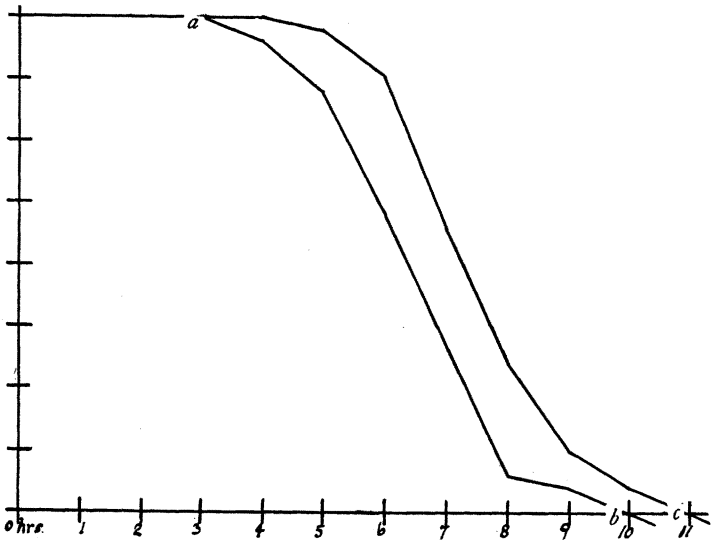

FIG. 1.

temperature and compared with that of worms which had been living at that temperature. All possible combinations of temperatures were used. The graphs represented by Figs. 1 and 2, from worms with one week "acclimation," with temperature

ordinates these values multiplied by the number of worms in each stage at each time interval, with the time intervals as abscissæ.

Since ten worms were used in each series, the highest possible numerical total, *i. e.*, the largest ordinate, will be 40, and will be attained when all the worms have reached Stage V., *i. e.*, are dead and disintegrated. The axis of ordinates is therefore divided into 40 spaces, each representing one unit of numerical value, and since the progress of death is most easily represented by descending curves, the ordinates are measured from above downward. Thus, for example, if we have a lot of ten worms which at a given time show the following groupings as regards stages of disintegration:

Stage I.	Stage II.	Stage III.	Stage IV.	Stage V
	2 worms	2 worms	4 worms	2 worms

The sum of the numerical values will be $2 + 4 + 12 + 8 = 26$, which is the ordinate for the curve at this time, and is to be measured downward from the zero point (see Fig. 1).

changes in both directions, are typical of the results of such experiments. Fig. 1 shows the susceptibility of two lots of worms, the experimental lot (medium-high) from a medium living temperature, represented by the curve *a–b*; the control (high-high) from a high living temperature, represented by the curve *a–c*; the susceptibility of both was tested at high. The position of the curve *a–b* well to the left of the curve *a–c* indicates that the worms which have been put suddenly from medium into high temperature to test are more susceptible to cyanide than the worms which have been living at that high temperature for a week previous to testing. Fig. 2 shows the same kind of experi-

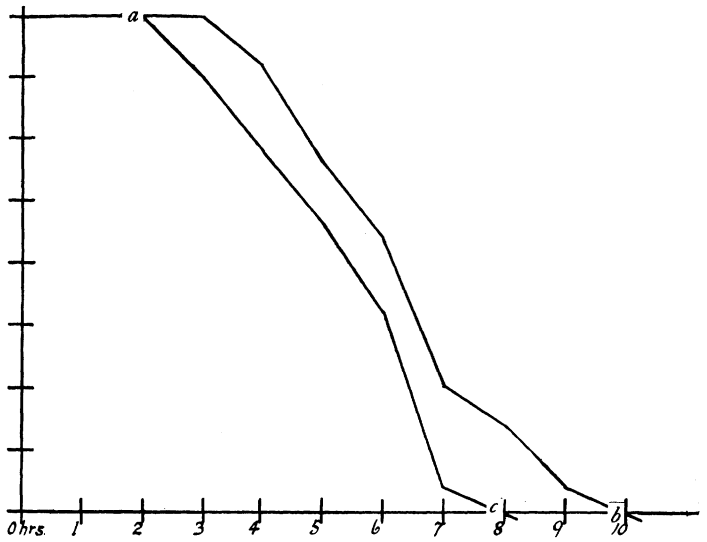

FIG. 2.

ment with opposite temperature change; here the experimental lot was living at high temperature a week, and its susceptibility, tested at medium temperature, compared with that of a control which had been living at that medium temperature. The curve *a–b* here represents the experimental (high-medium) lot, the curve *a–c* the control (medium-medium). The fact that the curve *a–b* lies well to the right of the curve *a–c* shows plainly that the worms which have been put into medium temperature to test are less susceptible to cyanide if they have previously been living at high for a week than if they have been in the medium temperature during that length of time.

It appears from the two graphs, then, that worms whose susceptibility is tested at a higher temperature than that at which they were living for a week are more susceptible than the controls; worms tested at a lower temperature than that at which they had lived a week previously are less susceptible than the controls.

Other data supporting these general conclusions are given below in Table I. Here are given the results of typical experiments differing from those pictured in Figs. 1 and 2 merely in the length of time that the experimental worms had lived in one temperature before being tested at the other. Some experiments with each direction of temperature change from the living to the testing one are given. Only the time of complete disintegration is recorded. In Table I. the first vertical column gives the series

TABLE I.

Series.	Experiment.		Testing Temperature.	Experimental Worms Died.	Controls.
	Acclimation Temperature.	Period of Acclimation.			
1	low	3 days	high	1 hr. before	high
2	low	4 days	medium	3 hrs. before	medium
3	low	4 days	high	3 hrs. before	high
4	medium	1 week	high	1 hr. before	high
5	high	1 week	medium	6 hrs. after	medium
6	low-	3 weeks	high	2 hrs. before	high
7	medium	3 weeks	low	2 hrs. after	low
8	low-	1 month	medium	2 hrs. before	medium
9	medium	1 month	low	2 hrs. after	low
10	high	2 months	low	10 hrs. after	low
11*	low-	3 months	medium	2 hrs. + before	medium

* In this case the difference between experimental lot and control is greater than is indicated in the table, because observation was concluded before the controls were completely dead and disintegrated.

numbers; the second column headed "acclimation temperature" gives the temperature the effect of which on a second change of temperature is to be tested. The third column, "acclimation period," gives the length of time during which the animals are kept at acclimation temperature. The fourth column, "testing temperature," gives the temperature at which the animals were tested. The fifth column shows whether the experimental lot is more or less susceptible than the control. And the last column shows the temperature at which the control worms were living

and are tested. For example, in No. 1, the experimental worms were kept three days in low temperature; then their susceptibility to KNC was tested at high temperature; and it was found that in general they died an hour earlier than the controls which had been living and were tested at high.

These eleven experiments give results similar to those indicated in the preceding graphs; worms tested immediately after they have been put into a higher temperature than that at which they have been living for a shorter or longer time show greater susceptibility to cyanide than those which have been living indefinitely at the higher temperature; those tested immediately after they have been put into a lower temperature than that at which they have been living for a shorter or longer time show a lower susceptibility to cyanide than those which have been living indefinitely at the lower temperature. In other words, worms brought into a given temperature after a period of exposure to another temperature show a difference in metabolic rate as indicated by susceptibility from the animals which have been living indefinitely at the given temperature.

B. *Short Time Acclimation.*

In the preceding section it has been shown that susceptibility to cyanide is modified by exposure to given temperatures for as short a time as three days. Further experiments were undertaken to determine whether or not such modification can be brought about in a still shorter period of time.

A few experiments were performed with worms which had lived but 36 hours at a particular temperature. These experiments presented nothing new and were therefore soon discontinued. Fig. 3 illustrates the general results of such experiments as were made. It is the record of 10 worms acclimated to 30° for 36 hours (curve *a–c*), compared in KNC at 16° with worms which had been living at 16° (curve *a–b*). This graph shows that worms living at high temperature for 36 hours are less susceptible to cyanide in low temperature than worms which have been living at that low temperature; that is, that even so short a period as 36 hours is long enough to modify the metabolic rate, though not to so marked a degree as longer time intervals.

A large number of experiments were performed to determine whether a 12 hours' exposure to a given temperature would alter

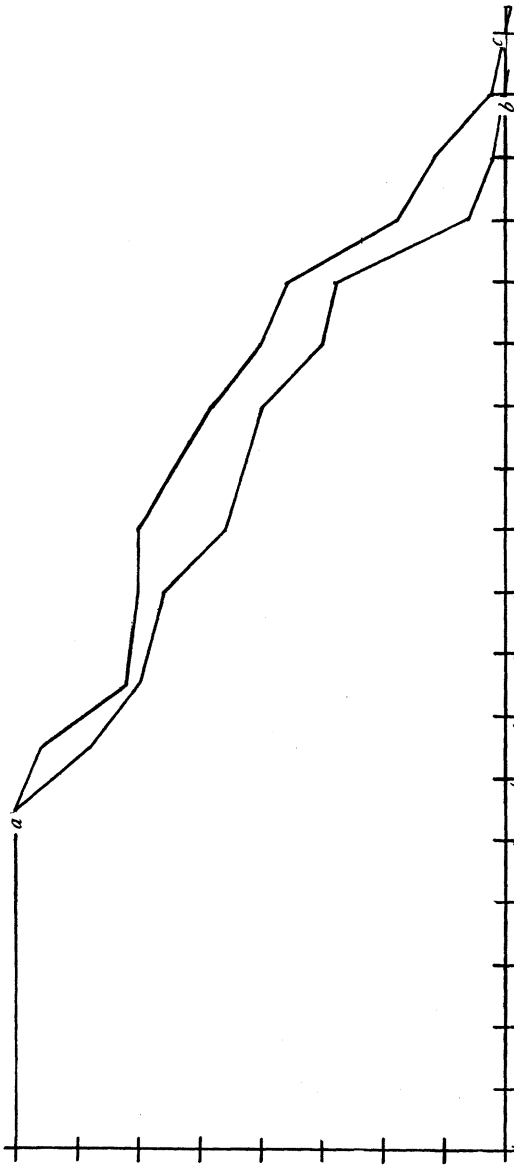

FIG. 3.

the susceptibility to cyanide. Worms from stocks kept for various lengths of time at one temperature were placed for

twelve hours in a different temperature; the susceptibility to cyanide was then compared at the second temperature with that of worms from the same stock which had not been exposed to the second temperature until the time of testing. Both lots were tested at the second temperature. If the worms show consistently different death rates it will be proof that time intervals as short as these few hours actually produce persistent modifications in metabolism. The results are more easily tabulated and considered in separate groups according to the direction of the temperature change.

In Table II. the experimental lots are brought into a tempera-

TABLE II.

Lowered Temperature.

No.	Comparative Susceptibility. Control Lots.		Experimental Lots.
1	high-low	>	high, 12 hrs. low
2	" "	>	" 12 hrs. "
3	" "	<	" 12 hrs. "
4	" "	=	" 12 hrs. "
5	" "	<	" 12 hrs. "
6	" "	<	" 12 hrs. "
7	" "	<	" 12 hrs. "
8	" "	<	" 12 hrs. "
9	" "	<	" 12 hrs. "
10	" "	>	" 12 hrs. "
11	" "	<	" 12 hrs. "
12	high-medium	<	" 12 hrs. medium
13	" "	=	" 12 hrs. "
14	" "	>	" 12 hrs. "
15	" "	<	" 12 hrs. "
16	medium-low	<	medium, 12 hrs. low
17	" "	<	" 12 hrs. "
18	" "	<	" 12 hrs. "
19	" "	<	" 12 hrs. "
20	" "	<	" 12 hrs. "
21	" "	<	" 12 hrs. "
22	" "	<	" 12 hrs. "

ture below that at which they have been living and remain there 12 hours before determination of their susceptibility is begun. The control lots, on the other hand, are brought into the lower temperature only when the determination of susceptibility begins. The table shows the difference in susceptibility between such

pairs of lots. In no. 1 for example, the control lot brought from high to low temperature at the time of susceptibility determination shows a susceptibility greater than that of the experimental lot which has been 12 hours at the low temperature before the susceptibility determination.

Of these twenty-two cases almost 73 per cent. show that 12 hours in a lower temperature than the living one *before* the addition of cyanide makes the worms more susceptible to cyanide than those which have been subjected to the depressing influence of the cold and of the cyanide simultaneously; in other words, the worms first brought into low temperature then subjected to cyanide die faster than those brought into low temperature and cyanide at the same time. Judging from these data a 12 hours' subjection to a lower temperature produces some degree of adjustment; in most cases the worms have apparently undergone some increase in metabolic rate during the twelve hours at the lower temperature. Just what the nature of this adjustment is had better be considered later when there are more data from which to judge. But that it is not merely a shock effect is evident from a comparison of the effects of acclimation periods of various lengths. In 12 hours the worms show less acclimation than in 36 hours, and in that period less than in three days, so that evidently the process is not ended in a period shorter than three days, which itself is surely too long a time for shock effect from a change of 10° to persist. The process then is gradual, covering a considerable period of time, which may range from three days to one week, by which time the acclimation is fairly complete and the new rate established.

The possible sources of error in these experiments account for most of the exceptional results quite readily. The series tested at low temperature all take so long even to begin disintegration that some degree of acclimation undoubtedly occurs in the period before death begins, which would bring the two lots very nearly to the same rate. This might well explain the inconsistency in no. 14, where a relation like the majority held until the last half hour at which time the condition became reversed. The fact that the series were not followed up to complete disintegration may explain the result of nos. 4 and 13. The worms

of nos. 1 and 2 belonged to a series which had previously been shifted several times from one temperature to another for very short periods of time; the repeated changes may have been so frequent as to check the effect of the last change. The result of the last of these six exceptions (a little over 27 per cent. of the total number of experiments), no. 10, can only be explained on the ground of possible individual variation.

Twenty-three experiments with temperature changes in the opposite direction were performed. Table III. summarizes the

TABLE III.

Raised Temperature.

No.	Comparative Susceptibility. Control Lots.		Experimental Lots.
1	low-high	>	low, 12 hrs. high
2	" "	>	" 12 hrs. "
3	" "	=	" 12 hrs. "
4	" "	<	" 12 hrs. "
5	" "	=	" 12 hrs. "
6	" "	>	" 12 hrs. "
7	" "	=	" 12 hrs. "
8	" "	=	" 12 hrs. "
9	" "	>	" 12 hrs. "
10	" "	>	" 12 hrs. "
11	low-medium	>	" 12 hrs. medium
12	" "	=	" 12 hrs. "
13	" "	<	" 12 hrs. "
14	" "	>	" 12 hrs. "
15	" "	=	" 12 hrs. "
16	" "	>	" 12 hrs. "
17	" "	>	" 12 hrs. "
18	medium-high	<	medium, 12 hrs. high
19	" "	<	" 12 hrs. "
20	" "	<	" 12 hrs. "
21	" "	>	" 12 hrs. "
22	" "	>	" 12 hrs. "
23	" "	>	" 12 hrs. "

results of such experiments. In these series the experimental lots are brought into a temperature higher than that at which they have been living and remain there 12 hours before determination of susceptibility is begun. The controls, as in Table II. above, are brought into the second temperature (in this case the higher one) only at the time when the determination of suscepti-

bility begins. The table shows the difference in susceptibility between the two lots. Thus no. 1, for example, shows that the susceptibility of a lot ("control") brought from low to high temperature only when the susceptibility determination is begun is greater than that of a lot ("experimental") which has been 12 hours at the high temperature before the susceptibility determination.

We can see from a glance at Table III. that there is much greater variability in the results in the raised temperature series than in the series with lowered temperature. Predicting results on the basis of the previous set of experiments, we would assume that after 12 hours in a higher temperature than the living one, before subjection to the KNC, the worms would be somewhat less susceptible than similar worms subjected to the new temperature and cyanide simultaneously. In other words, they should have become acclimated to some extent to this second change. Of the 23 series tested to determine this point ten gave the predicted result, six the opposite and seven showed no difference between the experimental and the control animals. Let us examine these to see whether a reasonable explanation for these very variable results can be found. One fact which may throw light on this question is the following: From examination of the graphs, which are not given here for lack of space, it is very evident that of the ten series which gave the result which we predicted from the findings in Table II., one showed a time interval of five hours between the death of the two series, one four hours' difference in time, and four two hours or very little less. The time intervals between complete disintegration of the two lots which showed the opposite type of result (those in which 12 hours' acclimation did not decrease susceptibility) were mostly shorter; the curves were closer together as a rule. Apparently, then, acclimation to raised temperatures is less rapid than acclimation to lowered ones since susceptibility seems to be much less altered by 12 hours at a temperature higher than that at which the worms have been living than it is by 12 hours at a temperature lower than that at which they have been living. In the case of the highest temperature used, this apparent retardation in the process of acclimation may perhaps be ex-

plained on the ground that this high temperature is close to the limit at which these animals can live at all after a sudden change; it has been found that they cannot live in a temperature above 30° unless the temperature is raised very gradually.

The susceptibility method shows then that exposure to a given temperature for even so short a time as 12 hours produces a change in physiological condition (Tables II., III.). When such a 12-hour period of exposure to a given temperature is followed by exposure to a higher temperature, the susceptibility determined at the higher temperature is higher than that of animals which have been living indefinitely at this higher temperature. The susceptibility determined at a temperature lower than that of the 12 hours' period is lower than the susceptibility of worms which have been living indefinitely at that lower temperature. With increase in the period of exposure to a given temperature this effect shows in general an increase (Table I.).

V. Carbon Dioxide Production.

The second method used for demonstrating the differences in metabolic rate at different temperatures consisted in a comparison of the CO_2 output of different lots. The measurement of CO_2 has long been used by physiologists as one of the best methods available for estimating the change in metabolic rate under experimental conditions. In these experiments carbon dioxide output was measured in two ways: by the colorimeter method and by the biometer.

A. *The Colorimetric Method.*

The colorimetric method was an adaptation of that used extensively by Haas in the laboratory of Plant Physiology of Harvard University. It consisted essentially in measuring the comparative CO_2 output of two lots of worms in terms of the color change in an indicator solution in which the worms were tested. The following indicators were tried:

Alizarin	Congo red	Phenolpthalein
Methyl orange	Benzo-purpurin	Phenolsulphone-pthalein
Neutral red	Liquid litmus	

Of these the turning point of the first three proved to be neither sufficiently close to the PH of the well water, nor sharply dif-

ferentiated enough, for the purpose of these experiments. Congo red and benzo-purpurin were not very satisfactory because they were taken up by the slime secreted by the worms so that the color in the solution itself became so varyingly diluted as to make accurate comparison uncertain. Liquid litmus and phenolpthalein were used to some extent; but by far the most satisfactory for the purposes of these experiments was the phenolsulphonepthalein used by Haas (1916) which combined all of the desirable qualities for a good indicator—non-toxicity, slowness of the penetration rate, great sensitiveness to very slight increase in H-ion concentration, and a suitable working range—from PH 8.6 to PH 6. The starting point for these experiments was of course the PH of the well water in which the worms lived in the laboratory—about PH 7.6; but this point is not very significant for the purposes of these experiments as no attempt was made to determine the absolute PH but rather the comparative changes in PH in terms of color differences.

In the earlier experiments equal numbers of worms of the same size were compared without weighing. Later, beginning with series XXV., and throughout the rest of the experiments, the worms were weighed and the weights recorded; and an attempt was made to put the excess weight now on one side, now on the other, so as to prevent the weight from being by any possibility the determining factor in the results. The worms were weighed in water at the temperature at which they had been living in each particular case. A small glass container with the water was first quickly balanced, and the worms were placed in this after a moment on filter paper to remove excess water. After weighing they were placed in pyrex tubes of standard volume; these were rinsed and then filled with indicator solution and sealed, with air excluded by paraffined corks, or, better paraffin plugs. The worms were then put into the new temperature away from the direct sunlight and all other strong illumination in order to preclude the possibility of stimulation from those sources. To avoid as far as possible the differences in motor activity resulting from differences in temperature the worms were decapitated, (except in a few cases indicated in the table), shortly before weighing. At any time from ten minutes

to twenty-four hours after decapitation there is almost complete inactivity in all the different temperatures, and the time between decapitation and experiment was always within these limits. That the animals do not excrete any appreciable amount of any non-volatile acid was shown by the fact that after the color change was produced by the worms the indicator solution could be brought back to the original color by shaking thoroughly with air; and there is no good reason to believe that they excrete any other volatile acid than CO_2.

The results obtained by this method are briefly as follows: first, worms brought from a low to a higher temperature show in the higher temperature a higher rate of metabolism as indicated by CO_2 production than that of the worms which have lived at the higher temperature; second, so far as the evidence goes worms brought from a higher to a lower temperature show a lower rate of CO_2 production than those which have been living at the lower temperature. Table IV. gives the results of 24 experiments in which worms acclimated to low and tested at medium temperature (experimental) are compared with worms which have been living indefinitely and are tested at medium temperature (control).

As can be seen at a glance the majority of these experiments gave very consistent results, extensive and beautiful evidence that worms acclimated to cold showed higher CO_2 production in medium temperature than worms acclimated to and tested at the medium temperature. Of the twenty-four experiments performed, seventeen, that is, 83 per cent., gave this result. The possibilities of experimental error here are: Observations over too short a period of time; too great a discrepancy in the weights of the two lots of worms; the inaccuracy of judgment due to the use of an unsatisfactory indicator. Of the four exceptions to the majority rule in this table, three, nos. 2, 6 and 21, can all be explained on one or the other of these grounds. No. 5 is explicable only as the result of individual variations in rate of CO_2 production, which are sometimes considerable.

The other two possible "raised-temperature" series—medium-high and low-high—gave results in the main like that of Table IV.; worms acclimated to a lower temperature than the one at

TABLE IV.

No.	Time Between Beginning of Experiment and First Noticeable Color Difference.	CO_2 Production. Control Lots.			Experimental Lots.	
1	1 hr.	medium-medium	>	low-medium		
	2½ hrs.	"	"	<	"	"
2	5 hrs.	"	"	>	"	"
3	2 hrs.	"	"	=	"	"
	4 hrs.	"	"	<	"	"
4	1½ hrs.	"	"	<	"	"
5	2½ hrs.	"	"	=	"	"
	3¼ hrs.	"	"	>	"	"
6	½ hr.	"	"	>	"	"
7	½ hr.	"	"	>	"	"
	6 hrs.	"	"	<	"	"
8	½ hr.	"	"	>	"	"
	1 hr.	"	"	<	"	"
9	1 hr.	"	"	<	"	"
10 (heads present)	½ hr.	"	"	<	"	"
11	½ hr.	"	"	<	"	"
12	3 hrs.	"	"	<	"	"
13 (heads present)	½ hr.	"	"	<	"	"
14	½ hr.	"	"	>	"	"
	1½ hrs.	"	"	=	"	"
	3 hrs.	"	"	<	"	"
15 (heads present)	3½ hrs.	"	"	<	"	"
16	3½ hrs.	"	"	<	"	"
17	½ hr.	"	"	<	"	"
18	½ hr.	"	"	<	"	"
19 (heads present)	1½ hrs.	"	"	<	"	"
20	1½ hrs.	"	"	<	"	"
21	1 hr.	"	"	<	"	"
	2 hrs.	"	"	>	"	"
22	3 hrs.	"	"	<	"	"
23	¾ hr.	"	"	<	"	"
24	¾ hr.	"	"	<	"	"

which they were tested produced more CO_2 in the same length of time than similar worms acclimated to and tested at the higher temperature. The only exception in this group belonged to a series exceptional in character throughout—with no apparent reason for its non-conformity except individual variations in rate. As a check upon this one series three other series with the same temperature conditions were tried later. The same length of time in the living temperature was allowed, the worms were weighed very carefully and tested with the most delicate indicator; under these conditions the typical result was obtained.

Few experiments with temperature changes in the opposite direction—acclimation temperature higher than testing temperature—were performed. The least satisfactory of these were the "high-low" vs. "low-low" series. This was because it required so long a time for the worms to show any appreciable CO_2 production that a new acclimation may have occurred or at least begun to occur in that time. Series LXVI., 1, "high-low" for instance, showed no color change at all even in the controls for more than 24 hours. As the previous experiments had shown even a 12-hour period to permit of some degree of acclimation, it is evident that in the above case we are dealing with something in the nature of a second acclimation. This difficulty could have been obviated by the use of greater numbers of worms; but beyond a few experiments to illustrate this point, no further attempts were made with this temperature combination. The two series "high-medium" vs. "medium-medium" and "medium-low" vs. "low-low" showed in over 60 per cent. of the cases that worms kept at a lower temperature have a higher rate of CO_2 production than those which have been suddenly brought into that temperature from a higher one.

Before leaving the subject of the indicator method, a few preliminary attempts to check the short-time acclimation periods may perhaps be mentioned. Though not conclusive on account of their small number, they are at least suggestive. The experimental procedure was as follows: Three lots of worms of as nearly as possible equal weights were taken, two that had been at low temperature, a third, the control, from medium temperature. The heads from all three lots were cut off, but at such times that one of the low temperature lots stood for a number of hours—12 to 24—headless before the experiment was begun; immediately after decapitating, this lot was put into medium temperature, so that it had, each time, 12 to 24 hours in which to adjust to that temperature before its rate of CO_2 production began to be measured. This gave for comparison with the control, two lots of the "low-medium" series, one of which was thus tested at once at the time of change of temperature, the other only after it had been given 12–24 hours in which to begin the process of acclimation. From the previous experimental data with short-

time acclimation we would suppose that the lot given a number of hours' start would have begun a second acclimation to the new temperature and would consequently show a new lower rate, producing less CO_2 in the same length of time than the lot which was subjected to change of temperature simultaneously with the beginning of the experiment. As a matter of fact such was the case in a few experiments tried in the way described above, at any rate up to the sixth hour after setting up the experiment, and in one case for as long as twenty-four hours at least. With other combinations of temperature in the same direction, the results were consistent with the above. "Medium-high" and "low-high" lots showed slower CO_2 production if given twelve hours' start at the second temperature than if the CO_2 production was estimated at once upon change of temperature. Thus even these few tentative experiments give further evidence of an acclimation after only 12–24 hours.

B. *The Biometer Method.*

The findings as to carbon dioxide production by the colorimetric method are further confirmed by the results of experiments with worms in the biometer, an apparatus devised by Tashiro (1914) to show very minute amounts of CO_2 by the formation of crystals of barium carbonate on the surface of a drop of the hydrate, and used extensively by him in the study of nerve metabolism and to some extent by him and Child ('13*b*) in experiments on this same form, *Planaria dorotocephala*. The biometer can be used for comparative estimation of CO_2 production in *Planaria*, but the method is so delicate that the best results are obtained with single individuals except when the worms are very small. With larger numbers the barium carbonate forms too rapidly to permit of accurate comparison,—accordingly all the experiments reported here are the results of testing one worm against another. The worms to be tested were decapitated to decrease motor activity, were weighed (in all cases except the first one), then dried for a moment on filter paper and put into the biometer on small cover slips. The method of weighing was the same as that for the colorimetric CO_2 determination (see above, p. 293); as often as possible the

excess weight was put on alternate sides in duplicate experiments, and the position of experimental and control worms in the apparatus was alternated. It should be stated, of course, that only those temperature combinations could be employed in which the testing and the control temperatures were medium since that was the only temperature at which the biometer could be operated. The "low-medium" group gave the same results as the colorimetric experiments of Table IV. above, as far as tried out. But it is from the "high-medium" lot, the one furnishing the less complete records in the colorimetric estimations, that the data below are quoted, since they are characteristic both as regards the majority results and the exceptions. Table V. is a summary of the results of these experiments. Worms which had been living at high temperature were put into the biometer at medium and their CO_2 production as determined by $BaCO_3$ precipitation was compared at that temperature with that of worms which had been living at the medium temperature indefinitely.

TABLE V.

No.	Weight in Grams.		CO_2 Production.				Remarks.
	Experiment.	Control.	Control.			Experimental.	
1			medium-medium	>	high-medium		
2	3½	2	"	>	"	"	
3	4	4	"	>	"	"	
4	3−	2+	"	<	"	"	
5	5	4+	"	>	"	"	
6	2−	2+	"	>	"	"	
7	5	7	"	<	"	"	
8	3−	3+	"	>	"	"	Probably a leak.
9	8	8	"	>	"	"	
10	7	5+	"	<	"	"	
11	6.5	6+	"	>	"	"	Experiment fed daily.
12	7	6−	"	>	"	"	
13	3−	2+	"	<	"	"	Experiment fed daily.

The results in the majority of the experiments harmonize with what we should expect from previous experiments. Of the 13 experiments here, 8, that is, nearly 62 per cent., show that animals which have been living at a higher temperature produce CO_2 less rapidly, that is, they have a lower metabolic rate when tested out at a lower temperature than do animals which have

been living at that lower temperature. Of the other four experiments, one, no. 8 (with positive results) has been left out of account, as it seemed probable that there was a leak in the biometer. In considering the exceptions we must bear in mind that small differences in weight may be enough, when dealing with such small amounts of protoplasm, to account for variations. Among the exceptions, three, nos. 4, 7 and 13, have the excess weight on the experimental side, but this is not the case with the fourth, no. 12; so weight can hardly be considered the explanation. As earlier experiments had shown that it was hard to maintain high temperature stock at the usual size on tri-weekly feedings, it seemed worth while when the first few cases of non-conformity appeared, to consider whether the irregularity could be found to have a definite relation to the nutritive conditions. But after daily feeding of one stock for a month, the last three experiments, 11, 12 and 13, failed to support any such theory. The excess weight was always on the experimental side yet the results differed in the different experiments, with no direct relation to the feeding. The exceptions here are probably due to slight differences in motor activity and to individual variations. As has been said above, since single individuals are used the effect of such differences must be relatively great.

From data on carbon dioxide production, then, we may draw conclusions identical with those to which the data from susceptibility experiments lead us. Worms which have been living at a given temperature for even as short periods as 12 hours have undergone an alteration in metabolism shown in this case by an alteration in CO_2 production. This alteration results in the production of more CO_2 by worms whose living temperature was lower than the testing temperature than by worms whose living and testing temperatures were the same (higher); and less CO_2 production by worms whose living temperature was higher than that at which they were tested than by worms whose living and testing temperatures were the same (lower) temperature.

VI. Influence of Temperature on Head-Frequency.

A. *Direct Effect of Altered Temperature.*

1. *During the Entire Period of Regulation.*—Before taking up the question of the effect of acclimation to certain temperatures

upon the head-frequency of regulating *Planaria*, we must consider: first, the significance of regulation experiments with this form; second, the factors concerned in head-frequency; and third, the direct effect of temperature on head-frequency.

In a study of regulation, whole worms are cut into certain pieces of equal size and allowed to remain for sufficient length of time to undergo growth and reorganization or redifferentiation. *Planaria dorotocephala* ordinarily reproduces asexually by fission, and worms above a certain size consist of two or more zooids which are distinguishable physiologically but not morphologically (Child, '11*c*). Since the head-frequency in the regulation of pieces has been found to vary according to the position of the piece with respect to the boundaries of the different zooids, it is desirable for the sake of uniformity to use pieces from a single zooid. The anterior zooid extending from the head to a short distance behind the mouth is the longest zooid, and most convenient for the purpose. Moreover, since head-frequency varies with length of piece and size of animal (Child,' 11*a*, '14*a*, '14*b*, '16), pieces of uniform length from animals of approximately the same size must be used. In the experiments reported below, three pieces, representing each one third of the length of the first zooid after removal of the head, are used and are designated *A*, *B* and *C*, in order from anterior to posterior (see Fig. 4). In all the experiments unless otherwise stated, the temperatures used were: "low," 10–12°; "medium" 20°; "high" 29°. During the period of regulation the water was changed frequently to guard against the accumulation of toxic substances. Professor

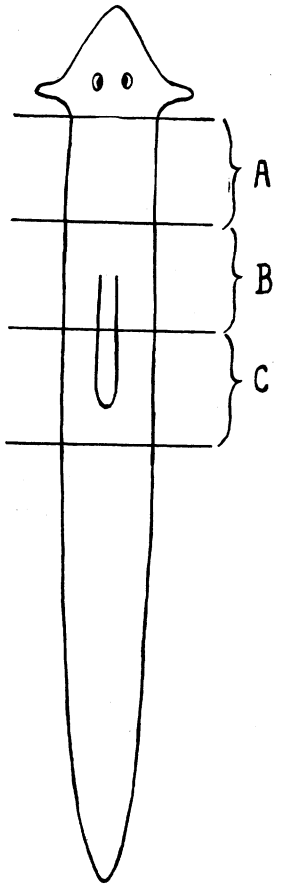

FIG. 4.

Child's morphological classification of the different degrees of cephalic differentiation between normal and headless ('11*c*) is followed in all the tabulations. Worms are classified as normal, teratophthalmic, teratomorphic, anophthalmic, and headless. In all experiments fifty pieces each were used and the numerical results are given in percentages unless otherwise stated.

On the basis of a wide range of experimental data, Child ('13*c*, '14*a*, '14*b*, '16) has attempted a physiological analysis of the factors concerned in head-frequency in this species, and his conclusions are adopted in this paper. His analysis is somewhat as follows: In each piece after section two regions are to be distinguished physiologically, one which can be designated *x*, the region at the anterior end of the piece from which the new head is formed; the other designated as *y*, consisting of the remainder of the piece (see Fig. 5). The region *x* undergoes more or less complete dedifferentiation as the result of section, its cells attain a more or less embryonic condition, and from this embryonic tissue the head gradually redifferentiates. The region *y*, on the other hand, shows stimulation through the nerves after section, but undergoes relatively little and only gradual dedifferentiation. Within any single individual, the more posterior the level of the body from which the piece is taken the greater the stimulation of *y* (Child, '14*a*). It has been shown that it is determined during this period of stimulation of *y* whether a piece shall form a head or not (Child, '14*b*) and that the more posterior the level of the body from which the piece is taken the lower the head-frequency (Child, '11*a*, '16).

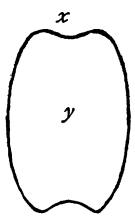

FIG. 5.

Head-frequency may be experimentally altered and controlled to a high degree (Child, '11*e*, '16) and the results of experiments along this line together with data noted above concerning the regions *x* and *y* have led Child to the conclusion that a new head is formed, not through correlation with and dependence upon the rest of the piece, but so to speak in spite of it. The differentiation of a head is determined by the embryonic cells of *x*, exactly as in the embryo arising from an egg, and the region *y* can only inhibit head development to a greater or less degree provided its

metabolic rate is sufficiently high in relation to that of x. All the experimental data agree in pointing to the conclusion that head-frequency increases with increase in rate x in relation to rate y, and decreases with the increase of rate y in relation to rate x. In other words, we may express head-frequency in the general formula head-frequency $= \frac{\text{rate } x}{\text{rate } y}$.

In order to show the direct effect of temperature on head-frequency, we may compare the head-frequency of pieces which have been living at a given temperature and undergo regulation at another temperature higher or lower than the first, with the head-frequency of pieces which have been living and undergo regulation at the first temperature.[1] Table VI. shows the effect on head-frequency of a rise from low to medium temperature, Table VII. the effect of a fall in temperature from medium to low, and Table VIII. the effect of a rise from medium to high. In each lot, A, B and C consist of 50 pieces, and the head-frequencies are given in percentages.

TABLE VI.

WORMS WHICH HAVE BEEN LIVING FOR SIX WEEKS AT LOW TEMPERATURE, REGULATING AT MEDIUM.

Series.	Living Temp.	Reg. Temp.	Piece.	Norm.	Teratophth.	Teratomorph.	Anoph. Th.	Headless.	Dead.
419 A.									
1.	Low	Medium	A	86	14				
			B	56	42	2			
			C	10	62	14	8	4	2
2. (control)	Low	Low	A	24	76				
			B		40	6	52	2	
			C		14	2	30	50	4

In Table VI., for example, where the first series of pieces have been living at low and undergo regulation at medium temperature, while the second series have been living and undergo regulation at low, the rise in temperature produces a very great increase in head-frequency. In the A pieces, with 24 per cent.

[1] These tables are made up from unpublished records of Professor Child which he has had the great kindness to put at my disposal. Practically all my own data along this line were rendered worthless by the chlorination of the city water.

TABLE VII.

WORMS WHICH HAVE BEEN LIVING FOR A NUMBER OF MONTHS IN THE LABORATORY AT MEDIUM TEMPERATURE, REGULATING AT LOW.

Series.	Living Temp.	Reg. Temp.	Piece.	Norm.	Teratophth.	Teratomorph.	Anoph. Th.	Headless.	Dead.
435									
1.	Medium	Low	A	44	50				6
			B	2	56	4	28	10	
			C		2	6	28	40	24
2. (control)	Medium	Medium	A	78	22				
			B		74	6	18	2	
			C		20	6	24	50	

normal and 76 per cent. teratophthalmic in the control, the rise in temperature for regulation alters this head-frequency to 86 per cent. normal and 14 per cent. teratophthalmic. In the *B* pieces the control shows no normals, 40 per cent. teratophthalmic, and 52 per cent. anophthalmic with 8 per cent. teratomorphic and headless together, while the rise in temperature alters this head-frequency to 56 per cent. normal, 42 per cent. teratophthalmic and only 2 per cent. of the lower types. In *C* pieces a similar increase in head-frequency results from a rise in temperature. Table VII. shows a great decrease in head-frequency

TABLE VIII.

WORMS WHICH HAVE BEEN LIVING FOR ALMOST TWO MONTHS IN THE LABORATORY AT MEDIUM TEMPERATURE, REGULATING AT HIGH.

Series.	Living Temp.	Reg. Temp.	Piece.	Norm.	Teratophth.	Teratomorph.	Anoph. Th.	Headless.	Dead.
572 A.									
1.	Medium	High	A	92	8				
			B	14	74	2	2	8	
			C	28	60	2	6	2	2
2. (control)	Medium	Medium	A	76	24				
			B		50	6	40	4	
			C	2	22	2	28	46	

as the result of regulation at a lower temperature than the living temperature. And Table VIII. shows an increase in head-frequency as the result of regulation in a temperature higher than the living temperature.

Let us consider briefly the significance of the data in the light of the interpretation of head-frequency suggested above. It will be seen that at a lowered temperature the head frequency throughout is lower than it would have been at the original temperature; at raised temperature it is higher than it would have been at the original temperature. Alteration of temperature then must affect x more than y; for if this were not the case we should expect head-frequency to remain unaltered. Apparently, then, the x cells are more susceptible to the direct effect of temperature changes than those of y.

This greater susceptibility of the x cells to temperature change is also indicated by the fact that at 4° C. the x region is incapable in almost 100 per cent. of the cases of giving rise to any head at all; although if the changes in temperature have not been too rapid the pieces may still remain alive at this temperature. Pieces kept for some five months at this temperature remained headless; but when brought into medium temperature gave rise to a high percentage of heads, many of which were normal. At this extremely low temperature life was maintained, but little or no growth of the physiologically younger cells of x occurred.

2. *During the First Few Hours of Regulation.*—Another series of experiments was conducted to see what would be the effect of temperatures applied for shorter periods of time than the whole time of regulation. These experiments were in a sense preliminary to acclimation experiments proper. They lie in intermediate position between those testing the direct effect of temperature during regulation and those testing the power of adjustment of the organism to temporary changes, and the extent to which such changes permanently alter the metabolic rate.

It has been found experimentally (Child, '14*b*) that whether a head shall be formed or not is determined within 3–6 hours after sectioning.[1] These experiments were repeated by me with like results (see Table IX. below).

We know that long pieces from standardized stock, such as a–x (Fig. 6), produce practically 100 per cent. normal heads. We also know that pieces such as a–b (Fig. 6) whose anterior

[1] Similar data have been worked out for *Lumbriculus inconstans* by Hyman ('16*a*).

ends are as nearly as possible at the same body level as the anterior ends of the long pieces give practically 100 per cent. headless forms. Moreover, we find that when a piece such as *a–b* has remained for a few hours as the anterior portion of the long piece *a–x* and is then isolated, it usually gives rise to a head. It is evident that at the anterior end of the piece conditions determining head formation have been so fixedly established during these few hours that they are not essentially altered by the later isolation of the short piece, although when the short piece is isolated at once head formation is inhibited.

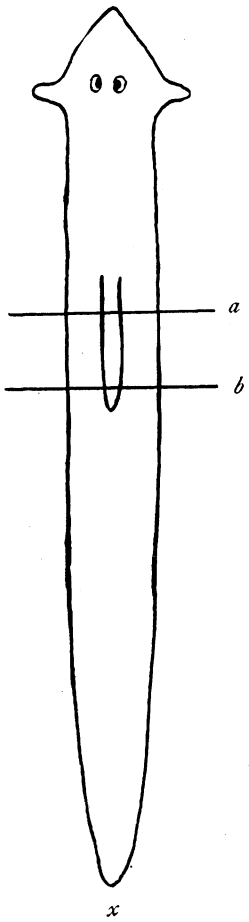

FIG. 6.

The experimental procedure is as follows: A lot (A) of 25 or 50 long pieces (*a–x*, Fig. 6) are cut and allowed to regulate, and head-frequency noted. A second lot (B), short pieces (*a–b*, Fig. 6), are likewise cut and allowed to regulate. These serve as controls. Several hundred long pieces (*a–x*, Fig. 6) are now cut, and from the anterior regions of these, lots (C, D, E, etc.) of short pieces (*a–b*, Fig. 6) are cut at different intervals following the first section. In this way the head forming region is left for a certain length of time as the anterior end of the long piece before it becomes the anterior end of the short piece. Table IX. gives the results of such a series of experiments.

Evidently the process of head determination then begins almost immediately after section and the conditions existing in the piece during the first few hours after section must constitute the most important factor determining the character of the result.

The controls *A* give 100 per cent. heads, whereas the controls

TABLE IX.

Lot.	Length of Time Between Two Cuts, at *a* and at *b*.	Per Cent. Heads.	Per Cent. Headless.
XXIV. A	Controls (*a*–*x*)	100	
B	Controls (*a*–*b*)	8	92
C	1 hour after first section	40	60
D	2 hours " " "	64	36
E	4 hours " " "	88	12
F	6 hours " " "	92	8

B give only 8 per cent. heads, and in the lots C, D, E, F, cut from the anterior ends of the long pieces at different intervals, it is evident that head-frequency increases with the length of the interval and that 6 hours' connection with the long piece is a sufficient interval to form a head in 92 per cent. of the pieces.

The demonstration of the occurrence of head determination within so short a period of time furnished good ground for the belief that temperature, acting through similar periods of time, would be able to produce an effect upon head-frequency. A few experiments along this line were undertaken. Worms from medium temperature were cut and immediately put for three hours into low temperature, after which they were left to regulate at medium temperature, and their head-frequency compared with that of a medium temperature series not subjected to low temperature. The results of such a series of 25 worms are given below in Table X. The death rate is somewhat high but the differences in head-frequency indicate some effect of the temperature change. Although these differences are slight, several repetitions of the experiments showed the same results, which are therefore to be considered typical.

TABLE X.

Lot.	Temp. for 3 Hrs. After Cutting.	Reg. Temp.	Heads, Per Cent.	Headless, Per Cent.	Dead, Per Cent.
XIV. 51	Medium	Medium			
A			98	2	
B			74	18	8
C			48	52	
XIV. 1	Low	Medium			
A			88	8	4
B			60	34	6
C			40	42	12

From the above table it will be seen that the effect of lowering temperature during the beginning of the period of regulation is of the same sort as the effect of a temperature continued during the whole period. Head-frequency is lowered throughout the series by the lowering of temperature.

B. *The Analysis of Acclimation by the Method of Head-Frequency.*

In the experiments of the preceding section, worms were taken from temperatures at which they had been living so long that there could be no doubt that acclimation is practically completed. In this section worms were used which had been living at a given temperature for shorter periods of time before putting into a new temperature to regulate. In this way an attempt was made to determine for how long a period worms must live in one temperature before acclimation to that temperature occurs to such a degree that it will affect the head-frequency at a new temperature.

The method consisted in keeping worms at a certain temperature for various lengths of time, from three days to three weeks. Then cutting A, B and C pieces for regulation at another temperature and comparing their head-frequency with that of pieces from animals living at the second temperature. A difference in head-frequency between the two lots will indicate that the first temperature has had some effect on the reaction to the second.

The following table gives a resumé of the results of such experiments with acclimation periods of from 3 days to 3 weeks.

Taking the first series as an illustration, the animals were kept at low temperature for three days and the *A*, *B*, *C* pieces when cut were placed in medium temperature for regulation. The head-frequency of this series was then compared with that of a control series which had been living and underwent regulation at the regulation (in this case medium) temperature. In this first series the head-frequency of the worms kept at low for three days before the pieces were cut shows in the *A* pieces a decrease, in the *B* and *C* pieces an increase in head-frequency as compared with the control. The data for the other series are recorded in the same way.

The table shows that the effect on head-frequency for shorter

TABLE XI.

Series.	Length and Temperature of Acclimation Period.	Regulation Temperature.	Head-frequency Compared with that of Worms Kept and Regulating at Regulation Temperature.		
			A.	B.	C.
1	low, 3 days	medium	decrease	increase	increase
2	low, 3 days	"	"	"	"
3	low, 3 days	"	"	"	"
4	high, 3 days	"	"	decrease	decrease
5	low, 3 days	"	increase	increase	increase
6	medium, 3 days	high	decrease	decrease	decrease
7	low, 4 days	medium	increase	increase	increase
8	medium, 4 days	high	decrease	"	"
9	low, 4 days	"	"	"	decrease
10	medium, 1 week	low	increase	——	"
11	medium, 1 week	high	"	increase	"
12	low, 1 week	medium	decrease	——	——
13	medium, 3 weeks	low	"	decrease	decrease
14	low, 3 weeks	medium	increase	increase	increase
15	low, 3 weeks	high	——	"	"
16	medium, 3 weeks	high	increase	"	"
17	low, 3 weeks	high	"	"	"
18	low, 3 weeks	medium	"	"	"
19	high, 3 weeks	medium	decrease	decrease	——
20	low, 3 weeks	medium	increase	increase	increase

periods up to one week is not entirely uniform; while with three weeks at the acclimation temperature, there is uniformity, head-frequency being higher than that of the control when the regulation temperature is higher than that of acclimation and vice versa. As might be expected, the head-frequency method of demonstrating the effect of acclimation is not as delicate as the other methods employed, and consequently longer periods of acclimation are required to give consistent and uniform results. Since the physiological methods have shown that at least some degree of acclimation to temperature occurs within a few hours (see pp. 307–8 above), it is evident that when pieces are brought into the new temperature for regulation the effect of any temperature to which they have been previously subjected will be more or less compensated by the beginning of acclimation to the new temperature even before head-frequency is determined. Consequently, this method can be expected to show only the larger effects resulting from longer periods of acclimation.

VII. DISCUSSION.

The experimental data on susceptibility, on CO_2 production as estimated both colorimetrically and by the biometer, and on

head-frequency in regulation all agree in indicating that when individuals of *Planaria dorotocephala* are subjected to changes in temperature which are not too extreme, two sorts of change in metabolic condition are distinguishable. The first of these is the change in oxidation rate which is the direct effect of the change in temperature, and is either an increase or a decrease in rate according as the change consists in a rise or a fall in temperature. The second is a gradual process of acclimation extending over days or weeks and apparently gradually approaching a limit. This process involves an alteration opposite in direction to the first, consisting in a gradual decrease in oxidation rate if the change is from a lower to a higher temperature, an increase if the change is from a higher to a lower temperature.

That this alteration in rate is a real acclimation and not merely a recovery from the shock of a change in temperature is indicated first by the fact that extreme changes are not necessary to bring it about; second, by the fact that it occurs when the temperature is raised the same number of degrees gradually as well as when it is raised abruptly, and third, that it only begins to be appreciable after twelve hours or more and may extend over a period of weeks. Recovery from any shock that might conceivably result from a change of 10° in temperature in an animal like *Planaria* might be expected to occur within a much shorter period of time than this acclimation process.

The acclimatory change in rate of oxidation, a decrease following rise, an increase following fall in temperature, represents the working of a regulatory mechanism which shows some resemblances in its results to the mechanism of temperature regulation in the warm-blooded animals. There, too, a rise in temperature brings about a decrease in rate of oxidation, and a fall, an increase in rate. It seems probable that a regulatory mechanism working in much the same way as that of *Planaria* will be found in other cold-blooded animals, and that such a general mechanism of acclimation to temperature in cold-blooded animals is the basis from which the temperature-regulating mechanism of warm-blooded animals is developed and elaborated.

The main differences between a general mechanism of acclimation to temperature such as that of *Planaria* and a temperature-

regulating mechanism such as that of warm-blooded animals seem to be that the former acts relatively slowly and only partially compensates the direct effect of temperature change; whereas the temperature-regulating mechanism acts very rapidly and maintains a uniform body temperature over a wide range of external temperature change.

Since the establishment of the fact that the usual temperature coefficient of velocity of chemical reaction for 10° C. within ordinary temperature range is between 2 and 3, and that the temperature-coefficients of many physical processes are of very different orders of magnitude, the temperature coefficients of various physiological processes have been determined on the assumption that such coefficients will show whether chemical or physical processes are primarily concerned. Snyder ('08) found that the velocities of nerve impulses follow van't Hoff's law for chemical reactions. Van Slyke and Cullen ('14) observed that the reaction rate of the enzyme urease is nearly doubled by every 10° rise in temperature between 10° and 40°, the average temperature coefficient being 1.91 within this range. Loeb and Wasteneys ('11) investigated the temperature coefficient for the rate of oxidation in newly fertilized *Arbacia* eggs and found it to be remarkably constant, about 2. And Lillie and Knowlton ('97) showed the temperature coefficient for the development of the egg of the frog to be of the same nature as that for chemical reactions. A great number of other similar cases might be quoted. Yet with the increase in knowledge along this line it has become increasingly evident that caution is necessary in generalizing from such data; for not only are some of the chemical and physical temperature coefficients almost identical, but as Bayliss ('15, p. 43) and others have pointed out, the physiological processes are processes occurring in heterogenous systems in which even when a chemical reaction occurs, other processes must also be concerned; and the velocity of the whole will be conditioned by that process the rate of which is lowest under the given conditions.

The experiments on temperature acclimation in *Planaria* show that in addition to the direct effect of temperature on the velocity of cèr ain physiological processes, there is a secondary effect

occurring gradually and proceeding in the opposite direction from the first; that is, after a change in temperature, the velocity of physiological processes must undergo a gradual progressive change apparently approaching a limit in a constant temperature. Such a change manifestly cannot be an effect of temperature on the velocity of chemical reaction, but must result from an action of the altered velocity and other temperature effects associated with it upon the constitution or condition of the heterogenous protoplasmic system. These changes in the system in turn condition further gradual alterations in the velocity of reactions even though the temperature remains constant.

The effect of temperature upon head-frequency suggests that the processes in the region x (Fig. 5), the cells of which undergo extreme dedifferentiation and rapid growth and are directly concerned in head-formation, are altered to a greater degree by change in temperature than those in the region y. Regulation at a higher temperature than that at which the animal has been living increases, and regulation at a lower temperature decreases head-frequency. Moreover, acclimation to a given temperature alters the reaction, as indicated by head-frequency, to another temperature (Table XI.). This table also shows that animals acclimated to a high temperature show a lower head-frequency, and animals acclimated to a low temperature, a higher head-frequency, than those brought into that temperature at the beginning of regulation. These effects are in general opposite in direction to the direct effects of temperature change. If head-frequency $= \frac{\text{rate } x}{\text{rate } y}$ (pp. 302–3), as various lines of experimental evidence indicate, it is evident; first, that changes in temperature must directly alter rate x more than rate y; and second, that acclimation to a given temperature preceding regulation produces its effect by altering the metabolic level at which the regulatory processes in the cells of x begin.

These conclusions are in complete agreement with other data on acclimation to temperature and to other conditions in *Planaria*. In general, direct susceptibility to change in conditions and also capacity for acclimation to conditions which are not too extreme vary with rate of oxidation (Child, '13*a*, '13*c*,

14*a*, 14*c*, '16). A physiologically young animal shows a higher susceptibility to change in conditions as indicated by direct effect, and also is able to become more rapidly and completely acclimated to changes which are not too extreme. The cells of *x* undergo extreme dedifferentiation after section and become essentially embryonic cells before giving rise to a head. In an isolated piece then they very soon become physiologically younger than the cells of *y*, and the higher susceptibility of *x* to temperature changes as well as the effects of acclimation are unquestionably associated with this change in physiological condition and increase in metabolic activity.

In short, the data on head-frequency not only indicate direct and acclimatory effects of temperature of the same sort as those indicated by susceptibility to cyanide and CO_2 production, but they also indicate that these temperature effects are not the same for cells or tissues in different physiological condition. Here again the difficulty of drawing definite conclusions as to existence of a temperature coefficient of physiological processes is evident, from the fact that the rate of increase in the velocity of reaction may vary with the physiological condition of the cells or tissues concerned.

VIII. Summary.

1. The susceptibility of *Planaria dorotocephala* to toxic concentrations of KNC increases with rise and decreases with fall in temperature.

2. When animals which have been living at one temperature are subjected to another a gradual change in susceptibility to KNC in the new temperature occurs. In animals brought from a lower to a higher temperature the susceptibility undergoes gradual decrease at the higher temperature, and in animals brought from a higher to a lower temperature the susceptibility undergoes a gradual increase at the lower temperature. These secondary changes in susceptibility are distinguishable after twelve hours' subjection to a new temperature, but extend over days or weeks, apparently gradually approaching a limit. These changes occurring in a temperature higher or lower than that at which the animals have previously been living represent a process of acclimation.

3. Estimations of CO_2 production by a colorimetric method and by the Tashiro biometer give essentially the same results as the susceptibility method. The increase in CO_2 production which occurs when animals are brought from a lower to a higher temperature is followed by a gradual decrease at the higher temperature; and the decrease in CO_2 production which occurs when animals are brought from a higher temperature is followed by a gradual increase in CO_2 production at the lower temperature.

4. Experiments on the effect of temperature on head-frequency in the regulation of pieces give results similar to those on susceptibility and on CO_2 production. Rise in temperature increases, fall in temperature decreases, head-frequency, but acclimation determines changes in head-frequency in opposite directions from those directly determined by change of temperature. Animals which have become acclimated to a high temperature show a lower head-frequency in regulation than those brought into that temperature at the beginning of regulation; and animals acclimated to low temperature show a higher head-frequency than those brought into that temperature at the beginning of regulation.

5. All the experimental data agree in indicating that, within the temperature range of the experiments, acclimation to a change in temperature upward consists in changes which manifest themselves physiologically as a gradual decrease in rate of metabolism or oxidation; and acclimation to a change in temperature downward consists in changes which manifest themselves physiologically as a gradual increase in rate of metabolism or oxidation.

6. The working of the regulating mechanism concerned in acclimation to temperature resembles as regards its action on metabolism the temperature-regulating mechanism of warm-blooded animals, but is very much slower and less effective as a compensatory mechanism than the latter. It may perhaps be regarded as representing the general basis from which a temperature-regulating mechanism has developed.

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